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(54) Title: GENE ENCODING FOR SYSTEMIC ACQUIRED RESISTANCE IN ARABIDOPSIS

(57) Abstract

The invention relates to genes which are related to the transmission of Systemic Acquired Resistance (SAR) in plants via the signal transduction pathway. A gene has been characterized as to its ability to encode for a protein which acts upstream from salicylic acid in the signal transduction pathway.

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GENE ENCODING FOR SYSTEMIC ACQUIRED RESISTANCE IN ARABIDOPSIS

TECHNICAL FIELD

This invention relates to a gene specific to the signal transduction pathway for systemic acquired resistance in plants.

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of prior filed copending U.S. Provisional Application No. 60/046,475 filed May 14, 1997.

BACKGROUND OF THE INVENTION

The signal transduction pathway in plants that leads to Systemic Acquired Resistance (SAR) involves a number of stages. The first stage is the induction or immunization stage, where initial infection of a leaf by a pathogen to which the plant is resistant results in a hypersensitive response (HR) leading to the formation of necrotic lesions. There are, however, some examples in which SAR occurs without necrosis. Cameron, et al., "Biologically induced systemic acquired resistance in *Arabidopsis thaliana*," *Plant J* 5:715-725 (1994); Keller, et al., "Physiological and molecular characteristics of elicitin-induced systemic acquired resistance in tobacco," *Plant Physiol* 110:365-376 (1996). The expression in the inoculated leaf of a set of pathogenesis-related (PR) protein genes, commonly referred to as SAR genes, occurs during this induction stage in tobacco and cucumber (Kuc', J., *Bioscience* 32:854-856 (1982); Ward, et al., "Coordinate gene activity in response to agents that induce systemic acquired resistance," *Plant Cell* 3:49-59 (1991))and in Arabidopsis (Uknes, et al., "Acquired resistance in Arabidopsis," *Plant Cell* 4:645-656 (1992)); Uknes, et al., "Biological induction of systemic acquired resistance in Arabidopsis," *Mol Plant-Microbe Interact* 6:692-698 (1993)). Accumulation of salicylic acid (10- to 50-fold increase) also occurs during this stage. Yalpani, et al., "Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco," *Plant Cell* 3:809-818 (1991); Malamy, et al., "Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection," *Science* 250:1002-1004 (1990); Metraux, et al., "Increase in salicylic acid at the onset of systemic acquired resistance in cucumber," *Science* 250:1004-1006 (1990); Uknes, et al., *Plant Cell* 4:645-656 (1992); Vernooij, et al., "Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction," *Plant Cell* 6:959-965 (1994).

It has been reported that a phloem-mobile signal may move from the necrotic leaf to the rest of the plant to establish SAR. Jennes, A. and Kuc', J., "Graft transmission of systemic resistance of cucumber to anthracnose induced by *Colletotrichum lagenarium* and tobacco necrosis virus," *Phytopathology* 69:753-

756 (1979); Guedes, et al., "Induced systemic resistance to anthracnose in cucumber as influenced by the location of the inducer inoculation with *Colletotrichum lagenarium* and the onset of flowering and fruiting," *Physiol Plant Pathol* 17:229-233 (1980); Tuzun, S. and Kuc', J., "Movement of a factor in tobacco infected with *Peronospora tabacina* Adam which systemically protects against blue mold," *Physiol Plant Pathol* 26:321-330 (1985).

A study using ^{18}O-labeling of tobacco mosaic virus (TMV)-inoculated tobacco leaves revealed that a large percentage of the salicylic acid in the systemic leaves is synthesized in the initially infected leaf, consistent with salicylic acid itself being the mobile SAR signal. Shulaev, et al., "Is salicylic acid a translocated signal of systemic acquired resistance in tobacco," *Plant Cell* 7:1691-1701 (1995). However, other studies using cucumber (Rasmussen, et al., "Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*," *Plant Physiol* 97:1342-1347 (1991)), or grafting experiments with transgenic tobacco containing the bacterial *NahG* salicylic acid hydroxylase gene (and therefore exhibiting drastically reduced levels of salicylic acid), have led to the suggestion that salicylic acid is not the mobile signal. For example, *NahG* rootstocks, when inoculated with TMV, are capable of producing a phloem-mobile signal that can be perceived in the wild-type scion, which then becomes resistant to subsequent pathogen challenge. Gaffney, et al., "Requirement of salicylic acid for the induction of systemic acquired resistance," *Science* 261:754-756 (1993). These results suggest that salicylic acid is not required in the induction/immunization phase of SAR.

The second stage in the signal transduction pathway that leads to SAR is the establishment stage, which involves the perception of the mobile signal in systemic leaves. It is characterized by the expression of the same set of SAR genes as induced around the primary necrotic lesion, as well as the accumulation of salicylic acid, although to lower levels than are induced in the primary leaf during the induction stage.

The final stage in the signal transduction pathway is the expression stage which occurs when the plant is challenged with a second, normally virulent,

pathogen and responds to that pathogen as if it were an avirulent one. Kuc', J., *Bioscience* 32:854-856 (1982).

Recent studies using plants with very low levels of salicylic acid due to the expression of the *NahG* salicylic acid hydroxylase gene product (Gaffney, et al., *Science* 261:754-756 (1993); Vernooij, et al., *Plant Cell* 6:959-965 (1994); Delaney, et al., *Science* 266:1247-1250 (1994)) have shown that salicylic acid is required not only in the establishment and expression stages of SAR, but also during incompatible HR interactions, and even during compatible interactions to limit disease spread. The Arabidopsis SAR-defective mutants nim1 (Delaney, et al., *Science* 266:1247-1250 (1994)) and npr1 (Cao, et al., "Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance," *Plant Cell* 6:1583-1592 (1994)) also show enhanced disease susceptibility to virulent pathogens, confirming the role of salicylic acid in general disease resistance. Mutant cpr1 plants (constitutive expressor of PR genes) with elevated levels of SAR gene expression and salicylic acid were shown to be resistant to normally virulent pathogens (Bowling, et al., "A mutation in Arabidopsis that leads to constitutive expression of systemic acquired resistance," *Plant Cell* 6:1845-1857 (1994)), providing further genetic evidence in support of the importance of salicylic acid accumulation in disease resistance.

As outlined above, a number of Arabidopsis mutants affected in their ability to establish SAR have been isolated. However, these mutants also have a reduced general defense response, and therefore, the lesion in these plants is not specific for SAR.

Lipid transfer proteins (LTP's) have also been identified as important in the establishment of SAR in plants. LPT's have been cloned from a number of plant species, and have been proposed to function in a range of processes including embryogenic development and epicuticular wax deposition (Pyee and Kolattukudy, "The gene for the major cuticular wax-associated protein and three homologous genes from broccoli (*Brassica oleracea*) and their expression patterns," *Plant Journal* 7: 49-59 (1995); Toonen et al., "AtLTP1 luciferase expression during carrot somatic embryogenesis," *Plant Journal* 12: 1213-1221 (1997)). In relation

to the present invention, LTPs have already been implicated in plant defense responses. Thus, LTPs from two monocot species were shown to possess antimicrobial activity (Molina et al., "Lipid transfer proteins (nsLTPs) from barley and maize leaves are potent inhibitors of bacterial and fungal plant pathogens," *FEBS Letters* 316: 119-122 (1993)), and transgenic tobacco and Arabidopsis genetically engineered to express a barley LTP were shown to exhibit enhanced tolerance against pathogens (Molina and Garcia-Olmedo, "Enhanced tolerance to bacterial pathogens caused by the transgenic expression of barley lipid transfer protein LTP2," *Plant Journal* 12: 669-675 (1997)). These lipid transfer proteins have not been shown to function in SAR signal transduction.

Previous screens for SAR mutants have bypassed the initial signal production steps involved in SAR by inducing SAR through the plant by spraying with 2,6-dichloroisonicotinic acid (INA), which has been shown to act at the same point as, or even downstream of, salicylic acid in the SAR signal transduction pathway.

Despite reports concerning the possible role of salicylic acid in SAR, and the reported correlation of SAR gene expression with SAR, there is virtually no information available on the molecular mechanisms that result in the induction, transmission and expression of SAR. Thus, there was a need for the isolation and characterization of the genes that are a part of the SAR signal transduction pathway, which encode for proteins which act upstream of the site of action of salicylic acid.

It has now been found that genes which code for proteins which act upstream from salicylic acid in the signal transduction pathway affect SAR in plants, and a method for isolating those genes is herein disclosed. In particular, the dir-1 gene, which encodes a novel lipid transfer protein which is involved in the signal transduction pathway, has now been isolated and characterized. The gene is useful as a reagent for determining the mechanisms involved in establishing SAR in plants, and for genetically engineering disease-resistance in plants. A method for genetic screening of SAR mutants has also been developed which allows for detection of SAR-defective mutants throughout the SAR signal transduction

pathway, and more specifically, for those SAR mutants which encode proteins which act upstream of where salicylic acid acts in the SAR signal transduction pathway. Proteins translated from the genes are useful for the production of antibodies to the proteins. The antibodies are useful as reagents in screening plants for examples of the SAR signal transduction genes.

SUMMARY OF THE INVENTION

The present invention is directed to a nucleic acid molecule encoding a protein capable of enhancing systemic acquired resistance in a plant. The protein acts upstream from where salicylic acid acts along the signal transduction pathway.

The invention is also directed to a vector nucleic acid comprising the dir-1 gene.

The invention further comprises plant cells transformed with a vector containing the dir-1 gene.

This invention further comprises a method for conferring systemic acquired resistance to a plant.

The invention also comprises a method for isolating SAR mutants which encode proteins which act upstream of salicylic acid in the signal transduction pathway.

These and other features, aspects and advantages of the present invention will become better understood with reference to the following description and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the nucleotide sequence of the dir-1 gene.

Fig. 2 depicts the amino acid sequence of the dir-1 lipid transfer protein.

DETAILED DESCRIPTION

The present invention involves the isolation of genes involved in SAR signal transduction which encode proteins which act upstream from where salicylic acid acts along the SAR signal transduction pathway. To isolate a gene involved in

SAR signal transduction which encodes a protein which acts upstream from where salicylic acid acts along the pathway, the following method is used: Using techniques as described in Example 1, wild type and T-DNA transformed plants are grown and observed for disease symptoms. SAR mutants are isolated, and SAR competence tests are performed as described in Example 1. The SAR mutants are then tested to determine the position of the mutant gene in relation to the point of salicylic acid action in the pathway as described in Example 1. DNA is isolated from those plants which are homozygous for a SAR mutant gene, and where the gene is found to encode a protein which acts upstream from the site of salicylic acid action along the pathway, probed to isolate a cDNA, and sequenced using techniques as described in Example 2. The isolated genes, such as the dir-1 gene, can be used as vectors to transform plants for improved disease resistance by restoring or enhancing SAR. The plants to which the invention can be applied include the commercially important forage legumes such as but not limited to alfalfa, large-seeded legumes (grain legumes) such as but not limited to soybeans, beans, and peas, but not limited to solanaceous species such as tobacco, potato and tomato, and monocots such as but not limited to corn, wheat, and rice. Transformation may be accomplished by any known means including Agrobacterium (Shargool, Peter D. and Ngo, That T., *Biotechnological Applications of Plant Cultures*, 1994, pp. 61-76.), biolistic process (Shargool and Ngo at p. 38) or electroporation. Transformed plants may be regenerated by standard protocols well known to those with average skill in the art, such as organogenesis from leaf discs or somatic embryogenesis. The transformed plants may be propagated sexually, or by cell or tissue culture.

The invention includes the expression of the proteins translated from the isolated genes such as the dir-1 gene. These proteins, such as the dir-1 lipid transfer protein (dir-1 LTP), are useful in developing antibodies to the protein. The antibodies are valuable reagents to be used in the process of screening plants for the expression of isolated genes such as the dir-1 gene. To isolate a protein from a SAR signal transduction pathway gene, the gene product of the isolated gene is expressed as described in Example 5. Antibodies to the protein are

produced as described in Example 6. The antibodies to the protein are used as reagents for screening of plants for expression of the isolated gene as described in Example 6.

This invention includes a method for screening SAR-defective mutants throughout the SAR signal transduction pathway, and, more specifically, for screening for those mutants of genes which encode proteins which act upstream from where salicylic acid acts along the pathway. After transforming plants and then observing those plants for disease symptoms as described in Example 1, SAR mutants are isolated. The SAR mutants are then tested to determine the position of the mutant gene in relation to the point of salicylic acid action in the pathway as described in Example 1.

EXAMPLE 1

Isolation and characterization of dir-1 mutant

Plant and Bacterial Growth Conditions

Arabidopsis thaliana ecotype Wassilewskija (Ws) and the Feldman T-DNA transformed seed pools (Arabidopsis Biological Resource Center at Ohio State University) were sterilized, plated on Murashige minimal organics media, then transferred to soil after ten days as previously described. Cameron et al., *Plant J* 5:715-725 (1994). Plants were placed in growth chambers at 22 to 24°C at a light intensity of 150 μE m^{-2} sec^{-1} with a nine hour photoperiod, and grown for 4-5 weeks. *Pseudomonas syringae pv tomato* (Pst) strains DC3000[pLAFR3] (virulent) and DC3000[pLAFR3+avrRpt2] (avirulent) (Whalen, et al., "Identification of *Pseudomonas syringae* pathogens of Arabidopsis and a bacterial locus determining avirulence on both Arabidopsis and soybean," *Plant Cell* 3:49-59 (1991)) and *Ps pv maculicola* M4[pLAFR3+avrRpml] (avirulent) (Debener, et al., "Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate," *Plant J* 1:289-302 (1991)) were grown as previously described. Cameron et al., *Plant J* 5:715-725 (1994).

Isolation of SAR Mutants

Four to five week old T-DNA mutagenized plants (M1 generation) were given an immunizing inoculation of avirulent Pst (10^7 cfu/ml) on one leaf per plant, then two days later, challenge inoculated on three other leaves with virulent Pst ($5x10^5$ cfu/ml). Plants were observed four to five days later and symptoms compared to control avirulent (SAR induced) and virulent treatments performed on ecotype Ws. Those plants showing disease symptoms were selected as potential SAR mutants and allowed to set seed. Between 30 and 40 progeny of the putative SAR mutants were rescreened in the M2 generation as described below.

Genetic Characterization of dir-1

A number of dir-1 M2 plants were backcrossed to wild-type Ws and the resulting F1, F2, and F3 progeny subjected to a SAR competence test (described below) in order to determine whether the dir-1 mutation was recessive or dominant to wild-type, and also if the dir-1 phenotype cosegregated with the T-DNA (kanamycin resistance marker).

SAR Competence Test

Both dir-1 and wild-type Ws plants were subjected to a number of infection treatments. The control treatment (M,M) consisted of a primary inoculation with 10 mM $MgCl_2$ on one leaf per plant, followed two days later by another inoculation with 10 mM $MgCl_2$ on four other leaves/plant. An avirulent treatment (M,A) consisted of a primary inoculation with 10 mM $MgCl_2$ on one leaf per plant, followed two days later by an avirulent inoculum (10^6 cfu/ml) on four other leaves/plant. A virulent treatment (M,V) consisted of a primary inoculation with l0 mM $MgCl_2$ on one leaf/plant, followed two days later by a virulent inoculum (10^6 cfu/ml) on four other leaves per plant. A SAR treatment (A,V) consisted of a primary inoculation with avirulent bacteria (10^6 cfu/ml) on one leaf/plant, followed two days later with a virulent inoculum (10^6 cfu/ml) on four other leaves/plant.

Disease symptoms or their absence were noted for each treatment, and results confirmed by collecting leaf discs from each treatment for determination of *in planta* bacterial growth.

Quantification of Bacterial Growth *in planta*

Eight leaf discs (4.0 mm in diameter) were harvested from individual plants and macerated in 10 mM $MgCl_2$. Appropriate dilutions were made in 10 mM $MgCl_2$ and plated on King's B agar (King, et al., "Two simple media for demonstration of phycocyanin and fluorescein," *J Lab Clin Med* 44:301-307 (1954)) containing 100 mg/l rifampicin (Sigma Chemical Co., St. Louis, MO.) and 20 mg/l tetracycline (Sigma) to prevent growth of bacteria other than those used as inoculum.

Analysis of Salicylic Acid Levels

Salicylic acid was determined by grinding 0.5 g of Arabidopsis leaf tissue (20-30 leaves) in liquid N_2, then extracting in 50% ethanol plus 0.04% 2-mercapto-ethanol, followed by two volumes of 100% ethanol plus 0.04% 2-mercaptoethanol. The combined ethanol extracts were dried under N_2, until approximately 4 ml of water remained, then extracted two times with one volume of ethyl acetate. The ethyl acetate phase was dried under N_2, and the dried material resuspended in 200 μl of 100% methanol. Salicylic acid was quantified by reverse phase HPLC. Samples (20 μ1) were injected onto a 4.6 x 250 mm 5 μm C-18 silica column (J.T. Baker, Inc., Phillipsburg, NJ), equilibrated at ambient temperature with 95% 20 mM sodium acetate, pH 5.0, 5% methanol, and run isocratically at a flow rate of 1.5 ml/min. Salicylic acid (retention time approximately 7 min) was detected using a scanning fluorescence detector (Model FP-920; JASCO, Easton, MD) with excitation at 315 nm and emission at 405 nm, as described by Yalpani, et al. (1991). The identity of salicylic acid peaks from representative samples was confirmed by comparison of the emission spectra with that of an authentic salicylic acid standard. Salicylic acid peaks were also confirmed by digesting a number of previously determined salicylic acid samples with salicylic acid hydroxylase (Sigma)

and demonstrating that the salicylic acid peaks disappeared after this treatment. Total salicylic acid (free salicylic acid + glucose conjugate) was measured by digesting 50 μl of the methanol extracts with β-glucosidase. The loss of salicylic acid during the extraction procedure varied from 36 to 50% and was corrected for during the calculation of salicylic acid levels (ng/g fwt).

RNA Extraction and Analysis

RNA was isolated from frozen leaf samples (3-4 leaves/sample) using the small scale procedure of Verwoerd, et al., "A small scale procedure for the rapid isolation of plant RNAs," *Nucleic Acids Res* 17:23 8 2 (1989). Samples of total RNA (5 μg) were separated by electrophoresis through formaldehyde-agarose gels and blotted to Hybond-N nylon membranes (Amersham Corp., Arlington Heights, IL) as described in Sambrook, et al., *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, pp. 7.43-7.46 (1989). Ethidium bromide (40 μg/ml) was included in the sample buffer in order to confirm equal sample loading under UV light after electrophoresis. Arabidopsis cDNA clones for PR-1, PR-2 and PR-5 (Uknes, et al., *Plant Cell* 4:645-656 (1992)) were ^{32}P-labeled by random priming using a Random Primer Kit (Amersham). Hybridizations and washes were carried out according to Church, G.M. and Gilbert, W., "Genomic sequencing," *Proc Natl Acad Sci USA* 81:1991-1995 (1984). A rDNA probe was used to reconfirm that each lane was loaded with similar levels of total RNA.

Isolation and Genetic Characterization of the Arabidopsis dir-1 Mutant

Of 11,000 T-DNA transformed (MI) plant lines screened, 200 putative SAR mutants were recovered. After retesting in the M2 generation, the dir-1 gene proved to be a true SAR defective mutant. In two separate SAR tests of M2 dir-1 plants, the SAR defective phenotype segregated with a ratio of 3:1 (chi square values $p>0.9$ and $p>0.8$) for SAR defective to SAR competent (wild-type) plants. These results indicate that the dir-1 mutation is dominant to the wild-type SAR gene function.

Three different M2 dir-1 plants were backcrossed to the wild-type Ws background in order to confirm the dominant nature of the dir-1 mutation. The progeny (approximately ten plants) of each cross were tested again for SAR competence (see Table 1 for data on bacterial growth *in planta*). All the progeny from each cross were SAR defective, confirming the dominant nature of the dir-1 mutation.

Table 1: Bacterial Growth *in planta* (cfu/leaf disc) for dir-1 x Ws F3 progeny[1]

Genotype	Treatment[2]				
	Control (M,M)	Avirulent (M,A)	Virulent (M,V)	SAR (A,V)[3]	SAR (A,V)[4]
Ws	10^2	$8x10^5$	$5x10^7$	$2.2x10^6$	$2x10^6$
dir-1 F2-a	$2x10^2$	$6.4x10^5$	$4.6x10^7$	$8.0x10^7$	$3.7x10^7$
dir-1 F2-b	$5x\ 10^2$	$8.7x10^5$	$2.8x10^7$	$3.2x10^7$	-

[1] Eight leaf discs (2 replicates) were collected from each treatment, the average of 2 replicates is presented.

[2] (M,M) - Primary inoculation - $MgCl_2$; 2nd challenge inoculation -$MgCl_2$

(M,A)- Primary inoculation - $MgCl_2$; 2nd inoculation - avirulent Pst; collect inoculated or uninoculated leaves after 2nd challenge.

(M,V) - Primary inoculation - $MgCl_2$; 2nd inoculation - virulent Pst; collect inoculated leaves after 2nd challenge.

(A,V) - Primary inoculation - avirulent Pst; 2nd inoculation - virulent Pst; collect inoculated leaves after 2nd challenge.

[3] (A,V), A= PstavrRpt2.

[4] (A,V), A= PsmavrRpml.

The backcrossed dir-1 lines were allowed to set seed and were plated out (250-500 seeds) on kanamycin-containing media to determine if the SAR defective phenotype segregated with the kanamycin resistance gene present on the T-DNA to determine whether the dir-1 mutation was the result of insertion of the T-DNA

into the dir-1 gene. Seven backcrossed F2 lines were analyzed. Segregation ratios (kanamycin resistant to kanamycin sensitive) ranged from 7 to 3.3, indicating that there was at one time more than one T-DNA insertion per plant. If there were two functional copies of the T-DNA present in these lines, the ratio of kanamycin resistant to sensitive plants would be 15:1. These F2 lines (kanamycin resistant and sensitive) were transferred to soil, grown for four weeks, then tested for SAR competence by measurement of disease symptoms and bacterial growth levels. Very few of the kanamycin sensitive plants grew when transferred to soil, but for the most part, these plants were SAR competent. Plants from the F2 lines which grew on kanamycin ranged from 100% SAR defective as expected to 80% SAR defective, indicating that there was a functional kanamycin resistance gene in some of the progeny that was not linked to the dir-1 mutation.

DNA was isolated from both SAR defective, kanamycin resistant F2 plants and from SAR competent, kanamycin sensitive F2 plants. The DNA from each sample was digested with EcoR1 and subjected to electrophoresis, blotting, and probing with the plasmid pBR322, which is contained within the T-DNA construct. Errampalli, et al., "Embryonic lethals and T-DNA insertional mutagenesis in Arabidopsis," *Plant Cell* 3:149-157 (1991). A number of the F2 SAR defective, kanamycin resistant plants contained bands corresponding to the T-DNA that were not found in the SAR competent, kanamycin sensitive plants. A number of these dir-1 F2 kanamycin resistant plants were backcrossed again to wild-type Ws in order to eliminate any non-linked T-DNA sequences.

A number of the F2 families of the dir-1 x Ws backcross segregated 3:1 for kanamycin resistance. A SAR competence test was performed on individuals from two families that grew on kanamycin-containing media; if the T-DNA cosegregates with the dir-1 phenotype, all individuals are SAR defective. Each individual in both families (approximately 120) was SAR defective as measured by phenotype and bacterial quantification. These results confirm that the dir-1 mutant phenotype was segregating with kanamycin resistance and the T-DNA insertion. F3 seed from a number of F2 individuals was collected and was used to isolate dir-1 homozygous plants. All kanamycin sensitive F3 plants were homozygous wild-type

for dir-1, and expressed SAR. The genetic analysis therefore indicates that the dir-1 mutation is caused by the insertion of a T-DNA, facilitating the strategy of cloning the interrupted gene by polymerase chain reaction (PCR) techniques.

Rescue of the dir-1 Phenotype with INA

Spraying Arabidopsis with 2,6-dichloroisonicotinic acid (INA), can induce SAR to virulent fungal and bacterial pathogens. Uknes, et al., *Mol Plant-Microbe Interact* 6:692-698 (1993). INA is thought to act at the same point or downstream of salicylic acid in the SAR signal transduction pathway. (Vernooij et al., 1995). Therefore, dir-1 M2 plants were sprayed with INA and then challenged four days later with 10^6 cfu/ml of virulent bacteria; the plants were observed three days later and the symptoms recorded. The virulent control treatment (M,V) produced disease symptoms on both Ws and dir-1 plants, whereas the SAR treatment (A,V) produced disease in the dir-1 plants and resistance or SAR in the Ws plants. Both wild-type Ws and dir-1 plants that were sprayed with INA then challenged with virulent bacteria remained symptomless, indicating that INA induced SAR in dir-1 plants. This demonstrates that the dir-1 mutation is upstream of INA in the SAR signal transduction pathway. Substances such as, but not limited to, aspirin (acetylsalicylic acid), methysalicylate, and benzothiazoles which, like INA, mimic the effects of salicylic acid, may also be used.

SAR Gene Expression and Salicylic Acid Levels in the Arabidopsis dir-1 Mutant

In preliminary studies of dir-1 x Ws F2 progeny (kanamycin resistant, SAR defective) following inoculation with avirulent Pst (SAR treatment), expression of the SAR genes PR-1, PR-2 and PR-5 was reduced in the expression stage of SAR as compared to wild-type plants. However, salicylic acid accumulated to similar or higher levels than observed in wild-type plants (Table 2), consistent with earlier findings which show that salicylic acid accumulation is involved not only in SAR, but also in the hypersensitive response (HR) and general defense responses in Arabidopsis. Delaney, et al., *Science* 266:1247-1250 (1994); Cameron et al.,

unpublished). Moreover, SAR was not induced in the dir-1 mutant when a different avirulent bacterium (Psm M4 avrRpm1) was used as the immunizing primary inoculum, further indicating that the lesion in the dir-1 mutant plants is specific for the SAR signal transduction pathway.

Table 2: Free Salicylic Acid (ng/gfw) in Ws and dir-1 (F2 progeny)

Genotype	Control (M,M-inoc)[1]	Induction (M,A-inoc)[2]	Establishment (M,A-uninoc)[3]	Expression (A,V-inoc)[4]
Ws	95	1750	105	1186
dir-1 F2-a	93	2195	176	2500
dir-1 F2-b	-	1257	167	1017

[1] (M,M-inoc) - Primary inoculation - $MgCl_2$; 2nd challenge inoculation -$MgCl_2$; salicylic acid levels were determined for leaves collected from the 2nd inoculation.

[2,3] (M,A)- Primary inoculation - $MgCl_2$; 2nd inoculation - avirulent Pst; collect inoc or uninoc leaves after 2nd challenge.

[4] (A,V) - Primary inoculation - avirulent Pst; 2nd inoculation - virulent Pst; collect inoculated leaves after 2nd challenge.

EXAMPLE 2

Isolation and Characterization of the dir-1 Gene

Genomic DNA was isolated from leaves of a dir-1 homozygous line by standard procedures (Sambrook, et al., *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press (1989). Application of thermal assymetric interlaced (TAIL) PCR (Liu et al., "Efficient isolation and mapping of Arabadopsis thaliana T-DNA insert junctions by thermal assymetric interlaced PCR", Plant Journal 8: 457-463 (1995)) using primers specific for the right border (RB) of the T-DNA resulted in cloned sequences containing the RB but, instead of plant flanking DNA, inverted T-DNA sequences were found, indicating that a rearrangement had occurred within the T-DNA leading to an insert with two left borders (LBs). TAIL-PCR was then performed using primers designed to anneal to

the LB of the T-DNA. Two specific sequences were amplified, consisting of LB and plant genomic flanking sequences.

A combination of PCR and Southern blot analysis with genomic DNA from wild-type and dir-1 Arabidopsis indicated that the two sequences amplified by TAIL-PCR were contiguous in the genome of wild-type Arabidopsis, and were interrupted by the single T-DNA insertion. This insertion was in the 3' untranslated region of a gene, the open reading frame of which had significant sequence identity to that of a non-specific lipid transfer protein (LTP) from *Phaseolus vulgaris*. Sequencing the flanking DNA beyond the T-DNA insertion (away and out from the 3'-end of the LTP) revealed no open reading frame in over 1.1 KB of sequence. Furthermore, the LTP transcript levels were strongly reduced in total RNA preparations from the dir-1 line, suggesting that the T-DNA insertion does indeed interfere with the expression of the dir-1 gene. Probes from the genomic LTP sequence were used to isolate a cDNA encoding the complete LTP using standard methods (Sambrook, et al., *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press (1989)).

DNA Sequence Analysis

DNA was sequenced by the Sanger dideoxy sequencing method. (Sanger, et al., *Proc Natl Acad Sci USA* 74:5463-5467 (1977)). A *Taq* Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA) was used according to the manufacturer's protocol. The products were separated on a 6% polyacrylamide gel and the data processed by an ABI 373A automated DNA sequencer. All manipulations of raw data generated from the automated system were done on the PC Gene DNA analysis software (Intelligenetics, Mountain View, CA).

The nucleotide sequence is shown in SEQ ID NO: 1.

Evidence indicates that the Arabidopsis LTP encoded by dir-1 is a novel protein involved in signal transduction for establishing systemic plant immunity to disease. Firstly, the nature of the genetic screen targeted mutations in systemic rather than local responses, and this was borne out by the fact that the dir-1

mutation does not affect the local response to avirulent pathogens or the level of susceptibility to virulent pathogens in naive plants, as would occur if the dir-1 gene product was in itself antimicrobial. Secondly, inducers of SAR such as INA rescue the dir-1 phenotype, indicating that dir-1 is upstream of other signal functions such as npr1, mutations which eliminate responsiveness to INA (Cao et al., "Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance," *Plant Cell* 6: 1583-1592 (1994)). Thirdly, systemic induction of pathogenesis-related (PR) proteins and micro-HRs, molecular markers for the establishment of SAR in systemic leaves (Cao et al., "Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance," *Plant Cell* 6: 1583-1592 (1994); Alvarez et al., "Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity," Cell 92: 773-784 (1998)) is suppressed in dir-1.

The T-DNA insertion in the 3'-untranslated region of dir-1 leads to a reduction in corresponding transcript levels and therefore reduced levels of functional dir-1 LTP. This, in turn, blocks signal transduction from the primary infected leaf to systemic leaves of the plant. The identification of the dir-1 gene having dir-1 LTP as a translation product indicates that lipid intermediates act as systemic signals in SAR.

The amino acid sequence for the dir-1 LPT was derived using PC Gene software and is given in SEQ ID NO: 2.

EXAMPLE 3

Construction of a vector containing the dir-1 gene

Fragments containing the full length Arabidopsis dir-1 cDNA, or a functional homolog of dir-1 from another plant species, are cloned in a binary vector plasmid, for example in place of the GUS gene in the binary vector plasmid pBI121.1 (R. A. Jefferson, T. A. Kavanagh, M. W. Bevan, "GUS fusions: ß-glucuronidase as a sensitive and versatile gene fusion marker in higher plants," *The EMBRO Journal* 6: 3901-3907 (1987), under the control of the cauliflower mosaic virus 35S promoter (or any other constitutively expressed or inducible promoter

active in plant cells) and the nopaline synthase terminator (or any other transcriptional termination signal active in plant cells). Cloning and vector construction uses standard recombinant DNA techniques in *E. coli* strains HB101 or DH5a, performed according to (Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (2nd Ed), Cold Spring Harbor Laboratory Press, New York). Orientations of the insert are confirmed by restriction mapping, and the 5' ends of each construct confirmed by sequencing from an oligonucleotide primer from the 35S promoter or other promoters know to be used in the art.

EXAMPLE 4

Use of dir-1 gene vector in the transformation of plants and creation of transgenic plants

Tobacco and alfalfa plants are transformed with *A. tumefaciens* strain LBA4404 harboring the dir-1 gene construct by leaf disc methods. Transgenic tobacco plants (*Nicotiana tabacum* cv. Xanthi NF) are generated as described before (Rogers, et al., "Gene transfer in plants: Production of transformed plants using Ti plasmid vectors," *Methods Enzymol* **118**:627-640 (1986)), with regeneration under kanamycin selection. Transgenic alfalfa plants are generated from the transformation and regeneration of competent alfalfa cultivar Regen SY (Bingham, E.T., "Registration of alfalfa hybrid Regen-SY germplasm for tissue culture and transformation research," *Crop Sci* **31**:1098 (1991)), following a modified version of published procedures (Bingham, et al., "Breeding alfalfa which regenerates from callus tissue in culture," *Crop Sci* **15**:719-721 (1975)). Briefly, leaf discs from young trifoliate leaves are inoculated with a suspension of *Agrobacterium* harboring the binary construct and incubated on solid B5h plates (Brown, O.C.W. and Atanassov, A., "Role of genetic background in somatic embryogenesis in *Medicago*," *Plant Cell Tiss Organ Cult* **4**:111-122 (1985)) for four days (16 hours light at 24°C). The explants are then washed twice with water to remove bacteria and incubated for four more days on new B5h plates. Explants are then washed twice with water and transferred to selection plates (B5h plates

with 100 mg/L timentin (Smith-Kline Beecham, Philadelphia, PA) and 25 mg/L kanamycin (Sigma, St. Louis, MO)). Calli and occasional embryos appear after two weeks and are transferred to new selection plates, making sure the calli are spread out. Plates are incubated for another week to allow development of additional embryos. The calli and embryos are then transferred to B5 plates (no hormones, but with antibiotics as before). After two weeks, the calli and embryos are transferred to fresh B5 plates (with antibiotics). After one to two weeks, individual embryos are cultured on MS plates (Murashige, T., and Skoog, F., "A revised medium for rapid growth and bioassays with tobacco tissue culture," *Physiol Plant* **15**:473-497 (1962)) with antibiotics (50 mg/L timentin and 25 mg/L kanamycin); plantlets are formed within one to three weeks, occasionally with roots. These are transferred to plastic boxes (Magenta Corp, Chicago, IL) with MS agar media and antibiotics. Plants are maintained on MS media with antibiotics and propagated by cutting. Plants are also transferred to soil in the greenhouse.

EXAMPLE 5

Isolation of the dir -1 LTP

The Arabidopsis dir-1 gene product is expressed in *E. coli* SB221 cells as described (BS Shorrosh and RA Dixon, "Molecular cloning of a putative plant endomembrane protein resembling vertebrate protein disulfide isomerase and phosphatidylinositol-specific phospholipase C," *Proc Natl Acad of Sci* USA 88: 10941-10945). The expressed protein is resolved by polyacrylamide gel electrophoresis (Laemmli, U.K., "Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* 227: 680-685 (1970)). The protein band encoded by dir-1 is localized by staining with Coomassie brilliant blue, and regions from 12 gel lanes excised, cut into small fragments, destained to completion with 50% (v/v) isopropanol/3% (w/v) SDS overnight, rinsed with water, vacuum dried, ground in liquid nitrogen, and finally resuspended in phosphate buffered saline.

EXAMPLE 6

Production and use of antibodies to the dir-1 LTP protein

Antiserum is obtained by immunizing a female New Zealand White rabbit. The primary immunization contains approximately 30 μg of the dir-1 LTP in 2.7 ml of complete Freund's adjuvant, injected subcutaneously along the back at nine separate sites (300 ml per site). Booster injections containing dir-1 LTP from six excised gel lanes (approximately 15μg) in incomplete Freund's adjuvant are given at 4 and 6 weeks after the primary injections. The serum is stored at - 20°C.

The antibodies are used as a reagent to detect the dir-1 LTP in transgenic plants in a Western blot analysis. Leaf and stem proteins are extracted in 0.2 M borate buffer, pH 8.8 and subjected to denaturing polyacrylamide gel electrophoresis by standard procedures (Ausubel et al. 1994. *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., New York). Proteins are transferred to Immobilon-P membranes (Millipore, Milford, MA) in 25 mM Tris-HCl, pH 8.3, 192 mM glycine, and 20% methanol (Towbin et al. 1979. "Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications," *Proc Natl Acad Sci USA* 9:4350-4354). Membranes are blocked and probed with primary (anti-dir-1) and secondary antibodies, e.g., goat anti-rabbit IgG linked to a detection system such as alkaline phosphatase, in 3% BSA in TTBS (0.1% Tween-20 in Tris-buffered saline) (Ausubel et al., *Current Protocols in Molecular Biology* (1994)).

Although the present invention has been described in considerable detail with reference to certain preferred versions thereof, other versions are possible. Therefore, the spirit and scope of the appended claims should not be limited to the description of the preferred versions contained herein.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Gene Encoding for Systemic Acquired Resistance in Arabidopsis

(iii) NUMBER OF SEQUENCES: 2

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(F) ZIP: 75201-6507

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Hansen, Eugenia S.
(B) REGISTRATION NUMBER: 31,966
(C) REFERENCE/DOCKET NUMBER: 11137/03402

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 214-981-3315
(B) TELEFAX: 214-981-3400

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 439 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 36..341

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CAAACATATA GAAAAAGAGA GAGGAGGATA ATAAT ATG GCG AGC AAG AAA GCA          53
                                       Met Ala Ser Lys Lys Ala
                                         1               5

GCT ATG GCT ATG ATG GCG ATG ATC GTG ATA ATG GCT ATG TTG GTC GAT        101
Ala Met Ala Met Met Ala Met Ile Val Ile Met Ala Met Leu Val Asp
            10                  15                  20

ACA TCA GTA GCG ATA GAT CTC TGC GGC ATG AGC CAG GAT GAG TTG AAT        149
Thr Ser Val Ala Ile Asp Leu Cys Gly Met Ser Gln Asp Glu Leu Asn
        25                  30                  35

GAG TGC AAA CCA GCG GTT AGC AAG GAG AAT CCG ACG AGC CCA TCA CAG        197
Glu Cys Lys Pro Ala Val Ser Lys Glu Asn Pro Thr Ser Pro Ser Gln
    40                  45                  50

CCT TGC TGC ACC GCT CTG CAA CAC GCT GAT TTT GCA TGT CTT TGT GGT        245
Pro Cys Cys Thr Ala Leu Gln His Ala Asp Phe Ala Cys Leu Cys Gly
55                  60                  65                  70

TAC AAG AAC TCT CCA TGG CTC GGT TCT TTC GGT GTT GAT CCT GAA CTC        293
Tyr Lys Asn Ser Pro Trp Leu Gly Ser Phe Gly Val Asp Pro Glu Leu
                75                  80                  85
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GCT TCT GCT CTC CCC AAA CAG TGT GGT CTA GCC AAC GCC CCA ACT TGT        341
Ala Ser Ala Leu Pro Lys Gln Cys Gly Leu Ala Asn Ala Pro Thr Cys
             90                  95                  100


TAAAAGACTC TCTGTATACG TGTGTTTATG TTTTTTATTA CTCCTATTCT AAATATCGGA      401

TGTTATTAAT AAATCGTATT TTCTGCCAAA AAAAAAAA                              439
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Ser Lys Lys Ala Ala Met Ala Met Met Ala Met Ile Val Ile
  1               5                  10                  15

Met Ala Met Leu Val Asp Thr Ser Val Ala Ile Asp Leu Cys Gly Met
             20                  25                  30

Ser Gln Asp Glu Leu Asn Glu Cys Lys Pro Ala Val Ser Lys Glu Asn
         35                  40                  45

Pro Thr Ser Pro Ser Gln Pro Cys Cys Thr Ala Leu Gln His Ala Asp
     50                  55                  60

Phe Ala Cys Leu Cys Gly Tyr Lys Asn Ser Pro Trp Leu Gly Ser Phe
 65                  70                  75                  80

Gly Val Asp Pro Glu Leu Ala Ser Ala Leu Pro Lys Gln Cys Gly Leu
                 85                  90                  95

Ala Asn Ala Pro Thr Cys
             100
```

We claim:

1. A nucleic acid molecule comprising a region encoding a lipid transfer protein capable of SAR signal transduction.

2. The nucleic acid molecule of Claim 1 wherein said region comprises nucleotides 1 to about 439 as depicted in SEQ ID NO: 1.

3. A recombinant DNA molecule capable of functioning as a vector for plants comprising nucleotides 1 to about 439 as depicted in SEQ ID NO: 1.

4. A plant cell and plants transformed with a recombinant nucleic acid molecule substantially comprising nucleotides 1 to about 439 as depicted in SEQ ID NO: 1.

5. The plant cell and plants according to claim 4 wherein said recombinant nucleic acid molecule is integrated into the plant's chromosomal DNA.

6. A protein translated from the nucleic acid region of Claim 1.

7. A protein translated from the nucleic acid region of Claim 2.

8. A protein having an amino acid sequence as illustrated in SEQ ID NO: 2.

9. A vector comprising the nucleic acid molecule of Claim 1.

10. A vector comprising the nucleic acid molecule of Claim 2.

11. A transformed plant comprising the vector of Claim 10.

12. A transformed plant comprising the vector of Claim 11.

13. A method of conferring systemic acquired resistance to a plant comprising: transforming said plant with a vector comprising a nucleic acid region encoding a lipid transfer protein capable of directing systemic acquired resistance to a plant.

14. The method of Claim 12 wherein said nucleic acid region comprises nucleotides 1 to 439 as in SEQ ID NO: 1.

15. The method of Claim 12 wherein said protein has an amino acid sequence as illustrated in SEQ ID NO: 2.

16. A method of isolating SAR mutants which possess a region which encodes for a protein wherein said protein acts upstream from the point of salicylic acid action along the signal transduction pathway comprising:

a) transforming plants with an insertional mutagen;
b) observing for disease symptoms;
c) selecting for SAR mutants;
d) spraying said mutants with a salicylic acid like substance;
e) observing the phenotypes of said mutants; and
f) selecting for said mutants in which the wild type phenotype is restored as a result of said spraying.

17. An antibody capable of specifically binding the protein of claim 6, 7, or 8.

18. Substantially pure cDNA comprising a nucleotide region as shown in SEQ ID NO: 1.

19. Substantially pure cDNA encoding the amino acid sequence as shown in SEQ ID NO: 2 or functional equivalents thereof, the cDNA being substantially free of cDNA that does not encode the amino acid sequence shown in SEQ ID NO: 2 or functional equivalents thereof.

1/1

```
CAAACATATA  GAAAAAGAGA  GAGGAGGATA  ATAATATGGC  GAGCAAGAAA   50
GCAGCTATGG  CTATGATGGC  GATGATCGTG  ATAATGGCTA  TGTTGGTCGA  100
TACATCAGTA  GCGATAGATC  TCTGCGGCAT  GAGCCAGGAT  GAGTTGAATG  150
AGTGCAAACC  AGCGGTTAGC  AAGGAGAATC  CGACGAGCCC  ATCACAGCCT  200
TGCTGCACCG  CTCTGCAACA  CGCTGATTTT  GCATGTCTTT  GTGGTTACAA  250
GAACTCTCCA  TGGCTCGGTT  CTTTCGGTGT  TGATCCTGAA  CTCGCTTCTG  300
CTCTCCCCAA  ACAGTGTGGT  CTAGCCAACG  CCCCAACTTG  TTAAAAGACT  350
CTCTGTATAC  GTGTGTTTAT  GTTTTTTATT  ACTCCTATTC  TAAATATCGG  400
ATGTTATTAA  TAAATCGTAT  TTTCTGCCAA  AAAAAAAAA              439
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Fig. 1

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MASKKAAMAM  MAMIVIMAML  VDTSVAIDLC  GMSQDELNEC  KPAVSKENPT   50
SPSQPCCTAL  QHADFACLCG  YKNSPWLGSF  GVDPELASAL  PKQCGLANAP  100
TC
```

Fig. 2

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/US 98/09863

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C07K14/415 A01H5/00 C07K16/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOWLING, S.A., ET AL.: "A mutation in Arabidopsis that leads to constitutive expression of systemic acquired resistance" THE PLANT CELL, vol. 6, December 1994, pages 1845-1857, XP002075327 see page 1852, right-hand column, last paragraph - page 1853, left-hand column, paragraph 1; figure 8 ---	13
Y	DIETRICH, R.A., ET A.: "Arabidopsis mutants simulating disease resistance response" CELL, vol. 77, 1994, pages 565-577, XP002075328 see page 574, right-hand column --- -/--	13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 1998	03/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Maddox, A

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INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/US 98/09863

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PARKER, J.E., ET AL.: "Characterization of edsl, a mutation in Arabidopsis suppressing reistance to Peronospora parasitica specified by several different RPP genes" THE PLANT CELL, vol. 8, November 1996, pages 2033-2046, XP002074726 see page 2042, left-hand column, paragraph 2 ---	1-18
A	CENTURY, K.S.: "Genetic and molecular analysis of disease resistance in Arabidopsis thaliana" PHD THESIS. UNIVERSITY OF CALIFORNIA, BERKELEY, 1996, XP002074727 see page 26, paragraph 3 ---	1-18
A	CAMERON, R.K., ET AL.: "Biologically induced systemic acquired resistance in Arabidopsis thaliana" THE PLANT JOURNAL, vol. 5, no. 5, 1994, pages 715-725, XP002074728 cited in the application see the whole document ---	1-18
A	CAMERON, R.K., ET AL.: "Isolation of Arabidopsis mutants affected in the establishment of biologically induced systemic acquires resistance" JOUNAL OF CELLULAR BIOCHEMISTRY SUPPLEMENT, vol. 18A, 1994, page 92 XP002074729 see abstract X1-129 ---	1-18
A	RYALS, J.A., ET AL.: "Systemic acquired resistance" THE PLANT CELL, vol. 6, October 1996, pages 1809-1819, XP002075329 see the whole document ---	1-18
A	DELANEY, T.P., ET AL.: "Genetic dissection of acquired resistance to disease" PLANT PHYSIOLOGY, vol. 113, January 1997, pages 5-12, XP002074731 see the whole document --- -/--	1-18

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INTERNATIONAL SEARCH REPORT

Ir. ational Application No
PCT/US 98/09863

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	CHOI, D.-W., ET AL.: "Isolation of a root-specific cDNA encoding a ns-LTP-like protein from the roots of bean (Phaseolus vulgaris L.) seedlings" PLANT MOLECULAR BIOLOGY, vol. 30, 1995, pages 1059-1066, XP002074732 see the whole document	6-8
A	WO 92 20801 A (UNIV POLITECNICA DE MADRID) 26 November 1992 see the whole document	13
A	VERNOOIJ, B., ET AL.: "2,6-didichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid" MOL. PLANT-MICROBE INTERACT., vol. 8, no. 2, 1995, pages 228-234, XP002075162 see the whole document	16
P,A	WO 97 23617 A (SANDOZ LTD ;SANDOZ AG (DE); SANDOZ AG (AT); NIELSEN KLAUS KRISTIAN) 3 July 1997 see the whole document	1-3,6-8

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/09863

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9220801 A	26-11-1992	AU 655277 B	15-12-1994
		AU 1750092 A	30-12-1992
		BR 9205297 A	27-07-1993
		EP 0540709 A	12-05-1993
		HU 67779 A	28-04-1995
		JP 5508423 T	25-11-1993
		US 5446127 A	29-08-1995
WO 9723617 A	03-07-1997	AU 1377297 A	17-07-1997